氏　　名	Kishor（キショル）　Mazumder（マジムダ）
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学位論文名	Ursolic Acid Derivatives from Bangladeshi Medicinal Plant, Saurauja roxburghii: Isolation and Oxidative Derivatization for Cytotoxic Activity against Tumor Cell Lines （バングラデシュ薬効植物（Saurauja roxburghii）からのウルソール酸誘導体の単離と酸化誘導体化、ならびに選択的細胞毒性に関する研究）
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論文内容の要旨

Natural products are the most consistently successful source of biologically diverse compounds, and especially, the plant provides a large bank of rich, complex, and highly varied structures. Many higher plants contain the densely oxidized terpene metabolites, i.e., with carboxyl or hydroxyl groups, of which structural variants exhibit the diverse range of the activities. Alternatively, the core terpene structures could also be derivatized by the chemical oxidation in pursuit of enhancing the activity and/or modulating the target selectivity, i.e., against a specific tumor cell. This author investigated to isolate the natural products from the extracts of Bangladeshi medicinal plant, *Saurauja roxburghii,* a higher plant indigenous to south East Asia and some part of North America. By using the conventional extraction procedures, the author isolated the ursene-type pentacyclic triterpenes, i.e., ursolic acid **1**, corosolic acid **2**, 24-hydroxyl corosolic acid **3**, maslinic acid **4**, and a new ursene-type pentacyclic triterpene, 7,24-dihydroxyl ursolic acid **5**, from the chloroform fraction of the methanolic extract of the leaves of the plant (Figure 1). Structures of these five compounds were unambiguously determined by the extensive NMR and MS analyses, and

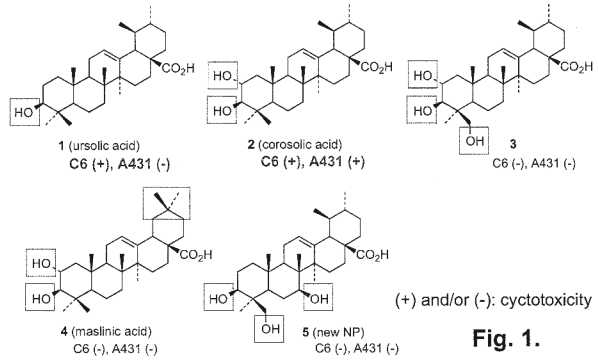

Fig. 1.

also by comparing with the literature data, if available.

They were then tested for the cytotoxicity against C6 rat glioma and A431 human skin carcinoma cell lines. Very interestingly, despite these five compounds have the same ursene-type pentacyclic triterpene core structures, only ursolic acid **1** and corosolic acid **2** showed the cytotoxicity at 10 μM concentrations. Furthermore, while the corosolic acid **2** showed the cytotoxicity against both the cell lines, the ursolic acid **1** exhibited the selective cytotoxicity against the C6 glioma cell. These results clearly showed that the position and numbers of hydroxyls on the terpene structure affect the activity and selectivity to the cancer cell lines.

Inspired by these results, the author challenged to diversify the hydrophobic ursene-type triterpene, the ursolic acid **1**, for further SAR studies, by applying the chemical oxidation using the "Ru"-porphyrin (Figure 2). Although the late-stage oxidation of the non-functionalized hydrocarbons is the recent trend in natural products synthesis, very few examples are reported to date, due to the scarcity of the oxidation reagents that efficiently activate the non-activated C-H bond. On the other hands, biomimetic oxidation using the porphyrin derivatives, i.e., the mimic of cytochrome P450, is intriguing opportunity for diversifying the core-structure of the ursolic acid **1**, although only one literature describes the related steroid oxidation by "Ru"-porphyrin in the presence of 2,6-dichloropyridine *N*-oxide. This author therefore prepared the seven "Ru"-porphyrin derivatives **a-g** from the commercially available tetraphenylporphyrin, which contain acid or different amines, in anticipating the additional auxiliaries affect the oxidation selectivity (Figure 2). While these "Ru"-porphyrins **a-g** gave **6-11** as the isolable products, their distributions were apparently affected by the auxiliaries. For examples, the parent "Ru"-porphyrin **a** gave the lactone **6** (25%) and the α-hydroxylated **7** (35%) as two major products, but the production of **7** was significantly decreased by the acid derivative **b** (4%), instead equally producing the other derivatives **8-10** (each about 10%). On the other hands, while the (*R*)-isomers **d** and **e** gave the α-hydroxylated **7** as the major product, the reactivity of the corresponding (*S*)-oxidants **f** and **g** was significantly reduced. Meanwhile, the ketone **11** was obtained only when the indane derivative **c** was utilized. This is the first observation that the auxiliaries on metalloporphyrin-based oxidants **a-g** gave profound effects on the oxidation reactivity and selectivity.

Out of these chemically oxidized compounds **6-11**, cytotoxicity was observed only for the lactone **6** against both A431 and C6 tumor cell lines; therefore the oxidation at C11 and C13 positions of ursolic acid **1**, gave the ursolic acid an additional cytotoxic activity against A431 tumor cell.

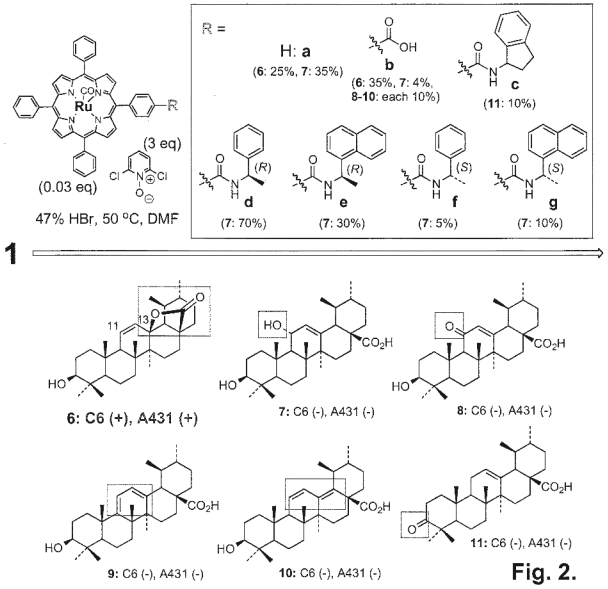

Fig. 2.

Thus, the author found that the cytotoxic activity and the tumor selectivity of the natural and the chemically oxidized ursolic acids are sensitively modulated by simply modulating the oxidation states on the core terpene structure. In this research, the chemical oxidation of natural terpenoids by "Ru"-porphyrins were also developed; the structural modification of the reagents significantly affects the oxidation out come. It is noted that chemically oxidized products **6-11** were all naturally occurring compounds, but were difficult to isolate from the *Saurauja roxburghii* as investigated initially, thus the chemical protocol can be complementary to the natural products isolation.

References

(1) K. Mazumder, E. R. O. Siwu, K. Tanaka, S. Nozaki, Y. Watanabe, K. Fukase, Ursolic Acid Derivatives from Bangladeshi Medicinal Plant, *Saurauja roxburghii*: Isolation and Cytotoxic Activity against A431 and C6 glioma Cell Lines, Phytochem. Lett., 4, 287-291, (2011).
(2) K. Mazumder, K. Tanaka, S. Nozaki, Y. Watanabe, K. Fukase, Chemical Oxidation of Ursolic Acid by "Ru"-porphyrins: Modulation of Cytotoxicity against Tumor Cell Lines, preparation for submission.

論文審査の結果の要旨

Kishor Mazumder は、バングラデシュ産薬効植物（Sauraja roxburghii）からのウルソール酸誘導体の単離ならびに構造決定を行い、5 種類の ursene 型 5 環状 triterpene, ursolic acid, corosolic acid, 24-hydroxyl corosolic acid, maslinic acid, と新規な ursene 型 triterpene である 7,24-dihydroxyl ursolic acid を同定した。これらについて C6 ラットグリオーマ細胞、A431 ヒト扁平上皮癌細胞に対する細胞毒性試験を実施し、構造活性相関について調べた。その結果 ursolic acid は C6 に選択的な細胞毒性、corosolic acid は C6, A431 の両方に対する細胞毒性を示し、その他の化合物はこれの細胞には作用しなかった。このように ursene 型 5 環状 triterpene の水酸化の度合いにより、生物活性が顕著に変化し、特定の癌細胞に選択的な細胞毒性を発現することを見出した。

続いて、天然物を出発物質として、様々な誘導体を合成し、それらについて構造活性相関を調べた。天然物の多くはその生合成過程において、種々のチトクローム 450 が作用することで酸化反応や水酸化反応を受ける。本研究ではチトクローム 450 ミミックとして、新たな触媒を探索することにした。触媒としてはすでに sp3 炭素の酸化に基づく酸化誘導体化が報告されている Ru-ポルフィリン錯体に着目し、ポルフィリンにカルボキシル基が導入されたもの、さらにカルボキシル基に光学活性アミンを結合させたものについて調べた。基質としては ursolic acid を用い、2,6-ジクロロピリジン N-オキシドを酸化剤に用いることで、水酸化を受けた化合物、水酸化と続くラクトン化された化合物、水酸基がケトンに酸化された化合物、ラクトンが脱離してアルケンとなった化合物など 6 種類の誘導体を得た。ここで用いる触媒に導入した置換基の構造や立体配置によってよって、反応性ならびに選択性が大きく異なることを見出した。得られた化合物群を同様の細胞毒性試験に供したところ、ラクトン化合物に C6, A431 の両方に対する細胞毒性が認められたが、他の化合物は不活性であった。このようにここでも ursene 型 5 環状 triterpene の微妙な構造上の差異により、活性が大きく変わることを見出した。

以上のように、Kishor Mazumder は ursene 型 5 環状 triterpene の抗腫瘍作用について構造活性相関を明らかにし、新規な酸化誘導体化法を見出すなど天然物化学の発展に貢献した。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。